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TURNOVER OF SERUM PROTEINS IN RATS WITH ANALBUMINEMIA

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SUMMARY: In the sera of a strain of rats with analbuminemia, the albumin concentration is less than a thousandth of that in normal rats. However, the total amount of serum proteins in the analbuminemic rats is almost the same as that in normal rats and no spots of additional proteins were observed on two dimensional gel electrophoresis. The half life of serum proteins as a whole in analbuminemic rats injected with [^{3}H]-leucine was 3.0 days, being similar to that of 2.9 days in normal rats. The serum of analbuminemic rats gave 3 distinct peaks of protein and radioactivity (P_1 to P_3), while those of normal rats gave an additional peak of albumin (P_4) on polyacrylamide gel electrophoresis. The half lives of P_1, P_2 and P_3 were 4.2, 2.4 and 2.1 days, respectively in analbuminemic rats and 2.5, 1.7 and 2.3 days, respectively in normal rats. The concentrations of P_1, P_2 and P_3 were 23.1, 19.9 and 20.9 mg/ml, respectively in analbuminemic rats and 4.4, 10.7 and 11.0 mg/ml, respectively in normal rats. The half lives of [^{3}H]-labeled rat albumin injected into the blood of analbuminemic and normal rats were 8.0 and 3.5 days, respectively.

INTRODUCTION

Serum albumin is considered to have important roles in the metabolism of the whole body, such as in maintenance of osmotic pressure, and as carriers of non-esterified fatty acid and thyroid hormone.

A case of lack of serum albumin was first reported in 1954 by Benhold et al. (1), and since that 13 other cases have been reported (2,3). These subjects were reported to be essentially healthy, but to have low levels of serum albumin, non-esterified fatty acid and triiodothyronine and high levels of triglyceride and cholesterol (3). These findings raise the question of the importance of serum albumin.

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Recently, Nagase and colleagues established a strain of rats completely deficient in serum albumin from a stock of Sprague-Dawley rats (to be published elsewhere). Analbuminemia was found to be an autosomal recessive phenotype. This strain of rats is a very good model for studies on human analbuminemia and on the function of serum albumin.

This paper reports studies on the turnovers of serum proteins in rats with analbuminemia and normal rats.

MATERIALS AND METHODS

Animals: Rats with analbuminemia were isolated from the stock of Sprague-Dawley rats of CLEA Japan (CLEA Japan, Kanagawa, Japan) (to be published elsewhere). The analbuminemic rats used in the present study were homozygous with respect to analbuminemia. Normal Sprague-Dawley rats of the same stock were obtained from CLEA Japan.

Chemicals: L-[3,4,5-^{3}H(N)]-Leucine (118.4 Ci/mmole) and Aquasol-2 were obtained from New England Nuclear (Boston, Mass., U.S.A.). Acrylamide and N,N'-methylene bis-acrylamide were products of Wako Pure Chemicals (Tokyo, Japan). DEAE-Sepharose CL6B was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). Hydroxylapatite was from BioRad (Richmond, Cal., U.S.A.). Ampholine (pH 3.5-10) was obtained from LKB (Bromma, Sweden). All other chemicals were of analytical grade.

Two dimensional gel electrophoresis: Two dimensional gel electrophoresis of serum was performed by a slight modification of the method of O'Farrel (4) with 2% Ampholine (pH 3.5-10) for the first dimension. Protein was stained with 0.2% Coomassie Brilliant Blue R-250 in a solution of 20% ethanol and 7% acetic acid in water.

Polyacrylamide gel electrophoresis: Polyacrylamide gel electrophoresis of serum was performed on a 5% acrylamide (4.86% acrylamide and 0.14% bis-acrylamide) slab gel in 0.1 M Tris-borate buffer (pH 8.7). After electrophoresis for 2 h, protein was stained with 0.2% Coomassie Brilliant Blue R-250 in a solution of 20% methanol and 7% acetic acid in water and destained in the same solution.

Densitometry was performed with a Joyce-Lebel Chromoscanner 200 by the refractory mode using a 620 nm filter. Protein in each peak was determined by densitometry.

Duplicate gels were cut into 2.5 mm slices and each slice was dissolved in 0.5 ml of 30% H_2O_2 by overnight incubation at 80°C. Radioactivity was counted after addition of 10 ml of Aquasol-2 in a Beckman LS8100 liquid scintillation counter.

Determination of radioactivity: Acid-insoluble radioactivity was determined by applying 20 µl of sample to a glass fiber paper (Whatman GF/C), washing the paper three times with 5% trichloroacetic acid and then with ethanol and ether and counting the radioactivity in Aquasol-2.

Determination of albumin: The concentration of serum albumin was determined by densitometry after electrophoresis and also by single radial immuno-

diffusion essentially by the method of Mancini et al. (5), using rat albumin (Miles Laboratories, Inc. Kankakee, Ill., U.S.A.) as a standard.

Preparation of [^{3}H]-leucine-labeled rat serum albumin: [^{3}H]-L-Leucine (0.6 mCi/rat) was injected intravenously into the tail vein of 5 normal male Sprague-Dawley rats weighing about 180 g. Two hours later, blood was collected from the abdominal aorta, and serum was obtained by centrifugation. The protein precipitated with 50-75% saturation of ammonium sulfate was dialysed against 10 mM sodium phosphate buffer (pH 7.0) and then applied to a DEAE-Sepharose CL6B column (2 x 20 cm) equilibrated with the same buffer. Material was eluted with a linear gradient of 0 to 0.5 M NaCl in 10 mM sodium phosphate buffer (pH 7.0). The fraction of eluate containing albumin was dialysed against 10 mM sodium phosphate buffer (pH 7.0) and applied to a hydroxylapatite column (2.6 x 5 cm) and the column was eluted with a linear gradient of 0.01 to 0.15 M sodium phosphate buffer (pH 7.0). The purified albumin gave a single protein band on SDS-polyacrylamide gel electrophoresis by the method of Leammli (6) and on immunoelectrophoresis (7) against anti-rat whole serum antiserum and had a specific radioactivity of about 50,000 cpm/mg protein.

Preparation of anti-rat serum proteins: Antibody against rat serum albumin was prepared by injecting the purified rat serum albumin described above into rabbits with Freund Complete adjuvant (Difco, Detroit, Mich., U.S.A.). The antiserum gave a single precipitin line against albumin on immunoelectrophoresis of whole rat serum protein. Anti-rat serum protein antiserum was prepared by injecting rat serum with Freund complete adjuvant into rabbits. Antisera against rat α_1-acid glycoprotein, α_1-X glycoprotein, transferrin, α_2-macroglobulin and γ-globulin were prepared by injecting the respective purified proteins with Freund complete adjuvant into rabbits.

RESULTS

Two dimensional gel electrophoresis of serum: In spite of the almost complete absence of serum albumin in analbuminemic rat serum, the total amount of serum proteins (75.7 ± 3.5 mg/ml) was almost the same as in normal rats (79.6 ± 7.7 mg/ml). As shown in Fig. 1, serum albumin was completely missing in analbuminemic rats, but the pattern of other proteins was quite similar to that in normal rats, except that, the amounts of four groups of proteins--transferrin, α_1-X glycoprotein, α_2-macroglobulin and heavy and light chains of immunoglobulin G--were considerably increased.

Metabolism of serum proteins: The metabolism of serum proteins was studied by labeling serum protein by injection of 200 µCi/100 g body weight of [^{3}H]-leucine through the tail vein. The fate of total acid-insoluble radioactivity in the serum after injection of [^{3}H]-leucine was similar in analbuminemic and

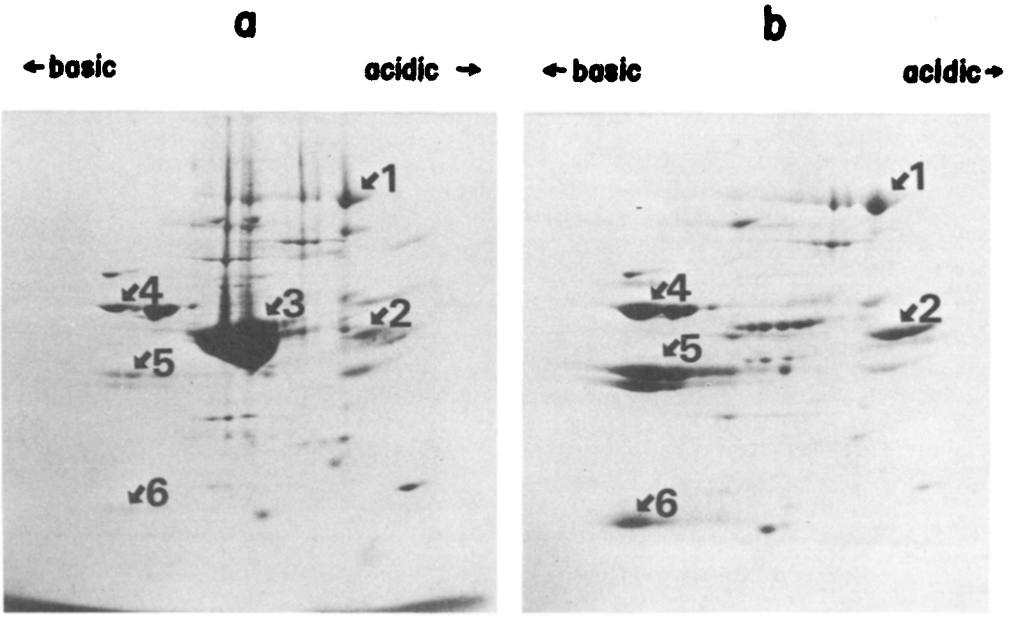

Fig. 1. Two dimensional gel electrophoretic patterns of serum proteins from normal rats (a) and rats with analbuminemia (b). 1; α_2-macroglobulin, 2; α_1-X glycoprotein, 3; albumin, 4; transferrin, 5; heavy chain of immunoglobulin G, 6; light chain of immunoglobulin G.

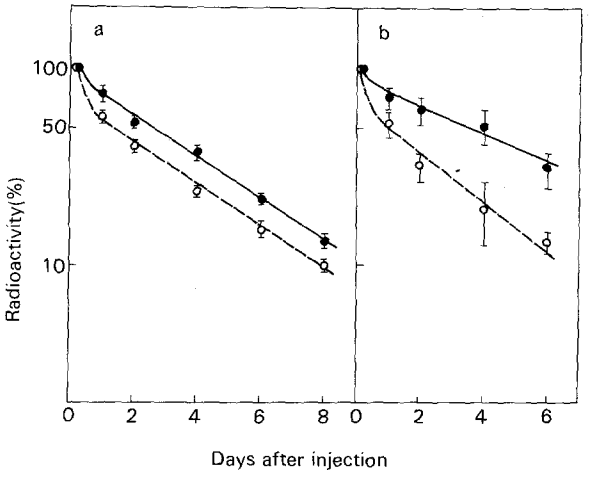

Fig. 2. Fate of [^{3}H]-labeled serum proteins. a; fate of serum acid-insoluble radioactivity in analbuminemic and normal rats. b; fate of radioactivities in P_1 in analbuminemic and normal rats. Values are means and standard deviations for 5 rats. Straight lines were obtained by the least square method. ●——●; analbuminemic rats, o---o; normal rats.

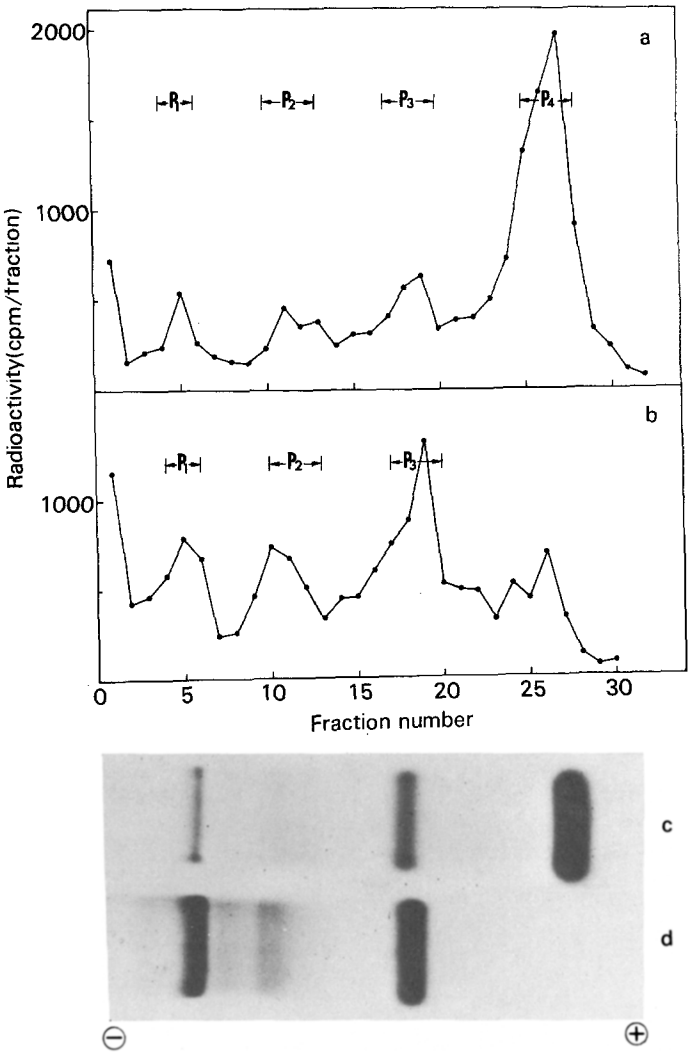

Fig. 3. Gel electrophoretic patterns of serum from normal and analbuminemic rats. Profiles of radioactivity were obtained 6 h after injecting [^{3}H]-L-leucine into normal rats (a and c) and analbuminemic rats (b and d).

normal rats, as shown in Fig. 2a. The radioactivity in the serum was maximal 4 and 2 h after injection of labeled amino acid in analbuminemic and normal rats, respectively, and these maximal values were defined as 100%. The half life of the serum proteins was 3.0 ± 0.1 days in analbuminemic rats and 2.9 ± 0.2 days in normal rats.

The labeled serum proteins were subjected to gel electrophoresis. Representative electrophoretic patterns of proteins and radioactivity on 5% poly-

Table 1. Half lives, concentrations and ratios of production of peak proteins

	$T_{1/2}$ (days)			Concentration (mg/ml)		
	Alb^-	Alb^+	Alb^-/Alb^+	Alb^-	Alb^+	Alb^-/Alb^+
P_1	4.2	2.5	1.7	23.1	4.4	5.3
P_2	2.4	1.7	1.4	19.9	10.7	1.9
P_3	2.1	2.3	0.91	20.9	11.0	1.9
P_4	-	2.6	-	0.0229[a]	34.2	0.0006

Values are means for 5 rats. Alb^+; normal rats, Alb^-; analbuminemic rats.

[a]Determined by single radial immunodiffusion.

acrylamide gel are shown in Fig. 3. Three peaks (P_1-P_3) of radioactivity and proteins were reproducibly obtained with serum from analbuminemic rats and four peaks (P_1-P_4) with that from normal rats. Immunoelectrophoresis against anti-sera to various serum proteins showed that P_1 mainly consisted of immunoglobulin G and α_2-macroglobulin, P_2 of transferrin, P_3 of α_1-X glycoprotein and P_4 in normal rats of albumin. One more peak of radioactivity was obtained at the origin of the gel, but this was not examined because its amount varied greatly in different experiments.

The fates of the radioactivity in P_1 in analbuminemic and normal animals are shown in Fig. 2b. The half lives and protein concentrations of the peaks are summarized in Table 1. Figure 2b and Table 1 clearly show differences in the half lives of P_1 and P_2 in the two groups of rats. However, no difference in the half life of P_3 was observed. In analbuminemic rats the serum concentration of P_1 was much higher than normal, and those of P_2 and P_3 were somewhat higher than normal.

Fate of injected rat serum albumin: Rats with analbuminemia and normal rats were given 3 mg/rat of [^{3}H]-labeled rat serum albumin (150,000 cpm) via the tail vein and acid-insoluble radioactivity in the serum was assayed at various times after the injection. This amount of serum albumin was too little to affect the concentration of serum albumin in normal rats. From the results

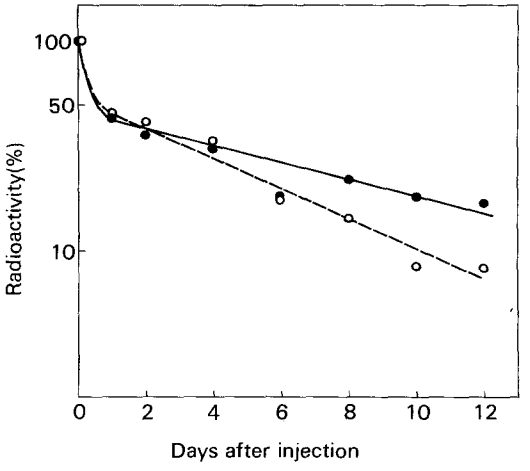

Fig. 4. Fate of injected [^{3}H]-labeled rat serum albumin in analbuminemic and normal rats. Values are means for 3 rats. Straight lines were obtained by the least square method. ●——●; analbuminemic rats, o---o; normal rats.

shown in Fig. 4, the half lives of albumin in analbuminemic and normal rats were calculated as 8.0 and 3.5 days, respectively.

DISCUSSION

In the analbuminemic rats studied in this work there was no albumin in the serum, but the total amount of serum proteins was almost normal. This normal level was due to increase in the concentration of other serum proteins, such as α_1-X glycoprotein, α_2-macroglobulin, transferrin and immunoglobulin G, without the appearance of any new serum proteins. The higher concentrations of these other serum proteins in rats with analbuminemia are probably due to increase in their production and/or decrease in their dergradation, because there was no significant change in the total plasma volume, assayed with Evans blue (8) and ^{51}Cr-labeling (9), or in the extravascular space, estimated by the sodium rodanate method (10) (data not shown), although the higher concentrations might be due to change in the distribution of serum proteins.

The serum concentration of P_1, which includes at least immunoglobulin G and α_2-macroglobulin, was 5.3 times higher in rats with analbuminemia than

in normal animals. Since the half life of P_1 in rats with analbuminemia was 1.7 times that in normal rats, the production of P_1 seems to be about 3 times higher in analbuminemic rats than in normal rats. In the same way, the production of P_2 in rats with analbuminemia was calculated to be 1.4 times higher than that in normal rats. The half life of P_3 in rats with analbuminemia was almost the same as that in normal rats, and thus its 1.9-fold higher concentration in their serum probably resulted from 1.9 times higher production of the protein. Since P_1, P_2 and P_3 are groups of heterogeneous proteins with different half lives, the apparent prolongation of their half lives in rats with analbuminemia could be a result of changes in their compositions. Further studies on this point are necessary. However, it seems likely that individual serum proteins have different turnover rates in rats with analbuminemia and normal rats, because injected serum albumin had significantly different half lives in these two types of rats.

Albumin has been considered to be an essential component of serum protein and it is one of the main proteins synthesized in the liver(11). However, rats with analbuminemia showed no significant pathological abnormalities, suggesting that other proteins effectively take over the functions of albumin in their serum.

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